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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 49/00, C07F 11/00, 15/00
C07D 471/10, C07K 15/28

A1

(11) International Publication Number: WO 93/11800

(43) International Publication Date: 24 June 1993 (24.06.93)

(21) International Application Number: PCT/US92/11003

(22) International Filing Date: 10 December 1992 (10.12.92)

(30) Priority data:
07/805,270 10 December 1991 (10.12.91) US

(60) Parent Application or Grant
(63) Related by Continuation
US 07/805,270 (CIP)
Filed on 10 December 1991 (10.12.91)

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(81) Designated States: AU, BR, CA, FI, HU, JP, KR, RU, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: BICYCLE-POLYAZAMACROCYCLOCARBOXYLIC ACID COMPLEXES, CONJUGATES, PREPARATION AND USE AS CONTRAST AGENTS

(57) Abstract

Complexes of bicyclopolyazamacrocyclocarboxylic acid with Gd, Mn or Fe ions are disclosed. The complexes can be covalently attached to a biologically active molecule, e.g. an antibody or antibody fragment, to form conjugates. The complexes and conjugates are useful as contrast agents for diagnostic purposes. Processes for preparing both the complex and conjugate are disclosed.

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"Bicycle-Polyazamacrocyclocarboxlic Acid Complexes, Conjugates, Preparation and Use as Contrast Agents"

This invention concerns complexes that contain as the ligand bicyclopolyaza-macrocyclocarboxylic acids, and conjugates thereof, for use as contrast agents in magnetic resonance imaging (MRI). Processes for preparing these complexes and conjugates are also provided. To better understand this invention, a brief background on MRI is provided in the following section.

Background

MRI is a non-invasive diagnostic technique which produces well resolved cross-sectional images of soft tissue within an animal body, preferably a human body. This technique is based upon the property of certain atomic nuclei (e.g. water protons) which possess a magnetic moment [as defined by mathematical equations; see G M. Barrow, Physical Chemistry, 3rd Ed., McGraw-Hill, NY (1973)] to align in an applied magnetic field. Once aligned, this equilibrium state can be perturbed by applying an external radio frequency (RF) pulse which causes the protons to be tilted out of alignment with the magnetic field. When the RF pulse is terminated, the nuclei return to their equilibrium state and the time required for this to occur is known as the relaxation time. The relaxation time consists of two parameters known as spin-lattice (T1) and spin-spin (T2) relaxation and it is these relaxation measurements which give information on the degree of molecular organization and interaction of protons with the surrounding environment.

Since water content of living tissue is substantial and variations in content and environment exist among tissue types, diagnostic images of biological organisms are obtained which reflect proton density and relaxation times. The greater the differences in relaxation times (T1 and T2) of protons present in tissue being examined, the greater will be the contrast in the obtained image [*J. Magnetic Resonance* 33, 83-106 (1979)].

It is known that paramagnetic chelates possessing a symmetric electronic ground state can dramatically affect the T1 and T2 relaxation rates of juxtaposed water protons and that the effectiveness of the chelate in this regard is related in part, to the number of unpaired electrons producing the magnetic moment [*Magnetic Resonance Annual* 231-266 (Raver Press

NY (1985)]. It has also been shown that when a paramagnetic chelate of this type is administered to a living animal, its effect on the T1 and T2 of various tissues can be directly observed in the magnetic resonance (MR) images with increased contrast being observed in the areas of chelate localization. It has therefore been proposed that stable, non-toxic paramagnetic chelates be administered to animals in order to increase the diagnostic information obtained by MRI [*Frontiers of Biol. Energetics* I, 752-759 (1978); *J. Nucl. Med.* 25, 506-513 (1984); Proc. of NMR Imaging Symp. (Oct. 26-27, 1980); F. A. Cotton et al., *Adv. Inorg. Chem.* 634-639 (1966)]. Paramagnetic metal chelates used in this manner are referred to as contrast enhancement agents or contrast agents.

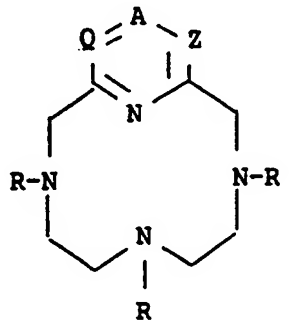
There are a number of paramagnetic metal ions which can be considered when undertaking the design of an MRI contrast agent. In practice, however, the most useful paramagnetic metal ions are gadolinium ($Gd^{+3}$), iron ($Fe^{+3}$), manganese ($Mn^{+2}$) and ($Mn^{+3}$), and chromium ($Cr^{+3}$), because these ions exert the greatest effect on water protons by virtue of their large magnetic moments. In a non-complexed form (e.g. $GdCl_3$), these metal ions are toxic to an animal, thereby precluding their use in the simple salt form. Therefore, a fundamental role of the organic chelating agent (also referred to as a ligand) is to render the paramagnetic metal non-toxic to the animal while preserving its desirable influence on T1 and T2 relaxation rates of the surrounding water protons.

Art in the MRI field is quite extensive, such that the following summary, not intended to be exhaustive, is provided only as a review of this area and other compounds that are possibly similar in structure. U.S. Patent 4,899,755 discloses a method of alternating the proton NMR relaxation times in the liver or bile duct of an animal using $Fe^{+3}$-ethylene-bis(2-hydroxyphenylglycine) complexes and its derivatives, and suggests among various other compounds the possible use of a pyridine macrocyclomethylenecarboxylic acid. U.S. Patent 4,880,008 (a CIP of U.S. Patent 4,899,755) discloses additional imaging data for liver tissue of rats, but without any additional complexes being shown. U.S. Patent 4,980,148 disclose gadolinium complexes for MRI which are non-cyclic compounds. C J. Broan et al., *J. Chem. Soc., Chem. Commun.*, 1739-1741 (1990) describe some bifunctional macrocyclic phosphinic acid compounds. C. J. Broan et al., *J. Chem. Soc., Chem. Commun.*, 1738-1739 (1990) describe compounds that are triazabicyclo compounds. I. K. Adzamli et al., *J. Med. Chem.* 32, 139-144 (1989) describes acyclic phosphonate derivatives of gadolinium complexes for NMR imaging.

At the present time, the only commercial contrast agent available in the U.S. is the complex of gadolinium with diethylenetriaminepentaacetic acid (DTPA-$Gd^{+3}$ - MAGNEVIST™ by Shering). MAGNEVIST™ is considered as a non-specific/perfusion agent since it freely distributes in extracellular fluid followed by efficient elimination through the renal system. MAGNEVIST™ has proven to be extremely valuable in the diagnosis of brain lesions since the accompanying breakdown of the blood/brain barrier allows perfusion of the contrast agent into the affected regions. In addition to MAGNEVIST™, Guerbet is commercially marketing a

macrocyclic perfusion agent (DOTAREM™) which presently is only available in Europe. A number of other potential contrast agents are in various stages of development.

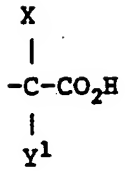
It would be advantageous if contrast agents were developed that could have site specificity for the tissue desired to be imaged, rather than non-specific/perfusion agents. The present invention is directed to just such novel complexes comprising a ligand that is a bicyclo-polyazamacrocyclocarboxylic acid of the formula



(I)

wherein:

R is



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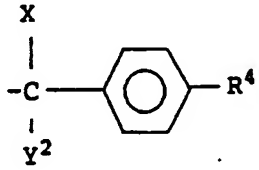
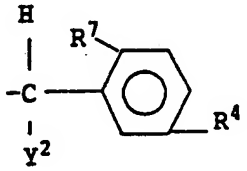


or



;

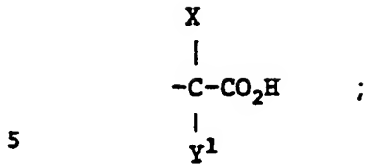
where:

X and $Y^1$ are independently H, OH or $C_1$-$C_3$ alkyl;

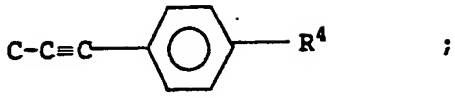
$Y^2$ is H or COOH;

$R^7$ is H, OH or $OCH_3$; and

$R^4$ is H, $NO_2$, $NH_2$, isothiocyanato, semicarbazido, thiosemicarbazido, maleimido, bromoacetamido or carboxyl; with the proviso that at least two R terms must be



A = CH, N, C-Br, C-Cl, C-OR$^1$, C-OR$^2$, N$^+$-R$^3$ X$^-$, or



R$^1$ = H, $C_1$-$C_5$ alkyl, benzyl, or benzyl substituted with at least one R$^4$;

R$^2$ is $C_1$-$C_{16}$ alkylamino;

R$^3$ is $C_1$-$C_{16}$ alkyl, benzyl, or benzyl substituted with at least one R$^4$;

R$^4$ is defined as before;

X$^-$ is Cl$^-$, Br$^-$, I$^-$ or $H_3CCO_2^-$;

Q and Z independently are CH, N, N$^+$-R$^3$ X$^-$, C-$CH_2$-OR$^1$ or C-C(O)-R$^5$;

R$^1$ and R$^3$ are defined as above;

R$^5$ is -O-($C_1$-$C_3$ alkyl), OH or NHR$^6$;

R$^6$ is $C_1$-$C_5$ alkyl or a biologically active material;

X$^-$ is defined as above; and

with the provisos that:

a) when Q, A or Z is N or N$^+$-R$^3$X$^-$, then the other two groups must be CH;

b) when A is C-Br, C-Cl, C-OR$^1$ or C-OR$^2$, then both Q and Z must be CH;

c) the sum of the R$^2$, R$^4$ and R$^5$ terms, when present, may not exceed one; and

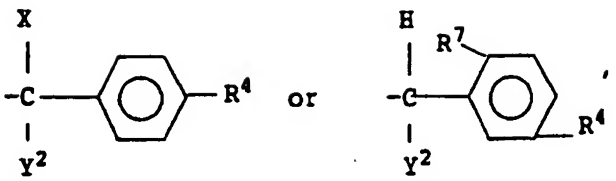
d) only one of Q or Z can be C-C(O)-R$^5$ and when one of Q or Z is C-C(O)-R$^5$, then A must be CH; and

complexed with a metal ion selected from $Gd^{+3}$, $Mn^{+2}$ or $Fe^{+3}$; or

pharmaceutically-acceptable salts thereof.

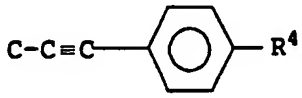
Bifunctional complexes of Formula (I) are desirable to prepare the conjugates of this invention. Such ligands must have at least one of R$^2$, R$^4$ or R$^6$ present:

one R term is



where X, $Y^2$, $R^4$ and $R^7$ are defined as above; or

A is C-$OR^1$, C-$OR^2$, where $R^1$ and $R^2$ are defined as above or



where $R^4$ is defined as above; or

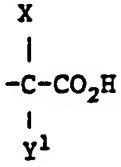
A is CH, and one of Q or Z is CH and the other is C-C(O)-$R^5$ or C-$CH_2$-$OR^1$, where $R^1$ and $R^5$ are defined as above; especially perferred are those ligands where $R^5$ is $NHR^6$, where $R^6$ is a biologically active material.

The ligands of Formula (I) are complexed with various metal ions, such as gadolinium ($Gd^{+3}$), iron ($Fe^{+3}$), and manganese ($Mn^{+2}$), and $Gd^{+3}$ being preferred The complexes so formed can be used by themselves or can be attached, by being covalently bonded, to a larger molecule, such as a dextran, a polypeptide or a biologically active molecule, including an antibody or fragment thereof, and used for diagnostic purposes. Such conjugates and complexes are useful as contrast agents.

The complexes and conjugates of this invention can be modified to provide a specific overall charge. For example, when the metal ion is + 3 the following can be obtained:

(A) an overall neutral charge - when

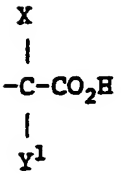
R is



and X and $Y^1$ are all equal to H;

(B) an overall + 1 charge - when

one of A, Q or Z is $N^+$-$R^3$ $X^-$, where $R^3$ and $X^-$ are defined as above; and the three R terms are

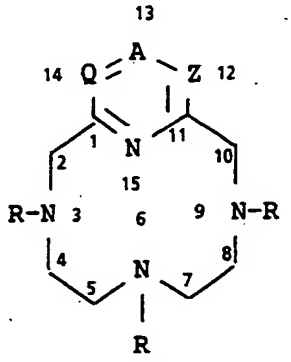


and X and $Y^1$ are all equal to H.

Both the complexes and conjugates may be formulated to be in a pharmaceutically acceptable form for administration to an animal.

Use of the ligands of this invention with other metal ions for diagnosis of disease states such as cancer is possible. The use of those complexes and conjugates is discussed in another copending application.

The complex has the ligand of Formula (I) numbered for nomenclature purposes as follows:



(I)

The present invention concerns development of contrast agents having a neutral or + 1 charge which enables site specific delivery of the contrast agent to a desired tissue. The advantage being increased contrast in the areas of interest based upon tissue affinity as opposed to contrast arising from non-specific perfusion which may or may not be apparent with an extracellular agent. The specificity of the ligand of Formula (I) may be controlled by adjusting the total charge and lipophilic character of the complex. The overall range of the charge of the complex is from + 1 to 0. For example, for a complex having a + 1 overall charge has heart uptake expected; whereas when the overall charge of the complex is 0 (thus neutral), the complex may have the ability to cross the blood brain barrier and normal brain uptake may be possible.

Tissue specificity may also be realized by ionic or covalent attachment of the chelate to a naturally occurring or synthetic macromolecule having specificity for a desired target tissue. One possible application of this approach is through the use of chelate

conjugated monoclonal antibodies which would transport the paramagnetic chelate to diseased tissue enabling visualization by MRI. In addition, attachment of a paramagnetic chelate to a macromolecule can further increase contrast agent efficiency resulting in improved contrast relative to the unbound chelate. Recent work by Lauffer (U.S. Patents 4,880,008 and 4,899,755) has demonstrated that variations in lipophilicity can result in tissue-specific agents and that increased lipophilic character favors non-covalent interactions with blood proteins resulting in enhancement of relaxivity.

Additionally, the present contrast agents of Formula (I) which are neutral in charge are particularly preferred for forming the conjugates of this invention since undesirable ionic interactions between the chelate and protein are minimized which preserves the antibody immunoreactivity. Also the present neutral complexes reduce the osmolarity relative to DTPA-$Gd^{+3}$, which may alleviate the discomfort of injection.

While not wishing to be bound by theory, it is believed that when a charged complex of the invention is made (e.g. + 1 for heart), the variations in that chelate ionic charge can influence biolocalization. Thus, if the antibody or other directing moiety is also specific for the same site, then the conjugate displays two portions to aid in site specific delivery.

The terms used in Formula (I) and for this invention are further defined as follows. "$C_1$-$C_3$ alkyl", "$C_1$-$C_5$ alkyl", "$C_1$-$C_{18}$ alkyl", include both straight and branched chain alkyl groups. An "animal" includes a warmblooded mammal, preferably a human being.

"Biologically active material" refers to, for example, a dextran, peptide, or molecules that have specific affinity for a receptor, or preferably antibodies or antibody fragments.

"Antibody" refers to any polyclonal, monoclonal, chimeric antibody or heteroantibody, preferably a monoclonal antibody; "antibody fragment" includes Fab fragments and $F(ab')_2$ fragments, and any portion of an antibody having specificity toward a desired epitope or epitopes. When using the term "radioactive metal chelate/antibody conjugate" or "conjugate", the "antibody" is meant to include whole antibodies and/or antibody fragments, including semisynthetic or genetically engineered variants thereof. Possible antibodies are 1116-NS-19-9 (anti-colorectal carcinoma), 1116-NS-3d (anti-CEA), 703D4 (anti-human lung cancer), 704A1 (anti-human lung cancer), CC49 (anti-TAG-72), CC83 (anti-TAG-72) and B72.3. The hybridoma cell lines 1116-NS-19-9, 1116-NS-3d, 703D4, 704A1, CC49, CC83 and B72.3 are deposited with the American Type Culture Collection, having the accession numbers ATCC HB 8059, ATCC CRL 8019, ATCC HB 8301, ATCC HB 8302, ATCC HB 9459, ATCC HB 9453 and ATCC HB 8108, respectively.

As used herein, "complex" refers to a complex of the compound of the invention, e.g. Formula (I), complexed with a metal ion, where at least one metal atom is chelated or sequestered; "conjugate" refers to a metal ion chelate that is covalently attached to an antibody or antibody fragment. The terms "bifunctional coordinator", "bifunctional chelating

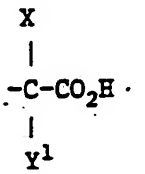
agent" and "functionalized chelant" are used interchangeably and refer to compounds that have a chelant moiety capable of chelating a metal ion and a moiety covalently bonded to the chelant moiety that is capable of serving as a means to covalently attach to an antibody or antibody fragment.

The bifunctional chelating agents described herein (represented by Formula I) can be used to chelate or sequester the metal ions so as to form metal ion chelates (also referred to herein as "complexes"). The complexes, because of the presence of the functionalizing moiety (represented by $R^2$, $R^4$ or $R^6$ in Formula I), can be covalently attached to biologically active materials, such as dextran, molecules that have specific affinity for a receptor, or preferably covalently attached to antibodies or antibody fragments. Thus the complexes described herein may be covalently attached to an antibody or antibody fragment or have specific affinity for a receptor and are referred to herein as "conjugates".

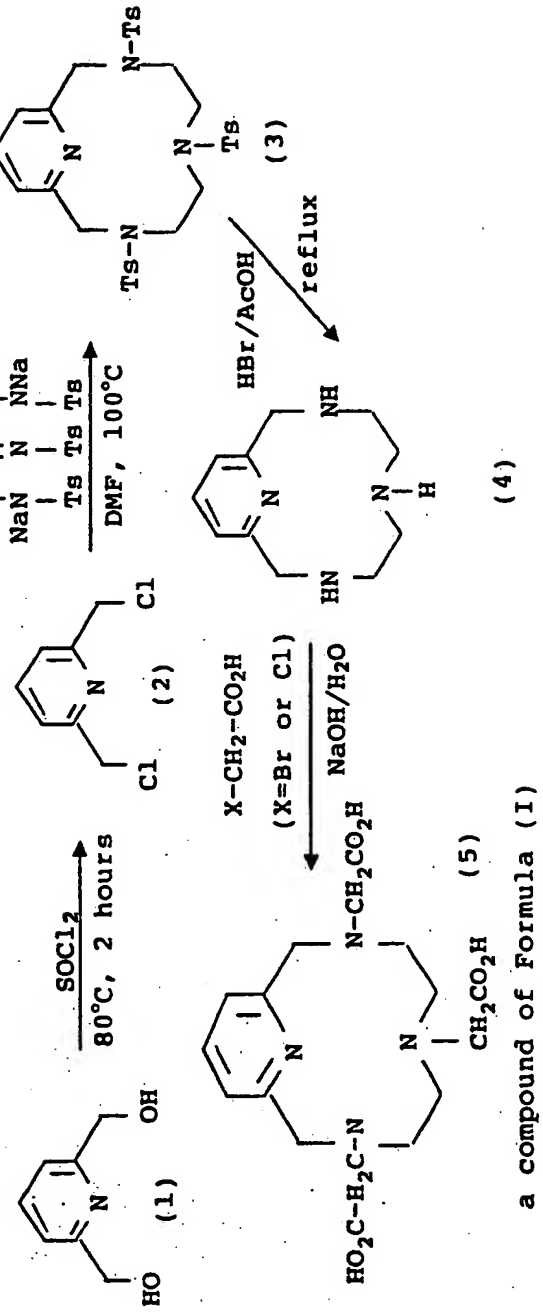
As used herein, "pharmaceutically-acceptable salt" means any salt or mixtures of salts of a complex or conjugate of formula (I) which is sufficiently non-toxic to be useful in therapy or diagnosis of animals, preferably mammals. Thus, the salts are useful in accordance with this invention. Representative of those salts formed by standard reactions from both organic and inorganic sources include, for example, sulfuric, hydrochloric, phosphoric, acetic, succinic, citric, lactic, maleic, fumaric, palmitic, cholic, palmoic, mucic, glutamic, gluconic, d-camphoric, glutaric, glycolic, phthalic, tartaric, formic, lauric, steric, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic acids and other suitable acids. Also included are salts formed by standard reactions from both organic and inorganic sources such as ammonium or 1-deoxy-1-(methylamino)-D-glucitol, alkali metal ions, alkaline earth metal ions, and other similar ions. Particularly preferred are the salts of the complexes or conjugates of formula (I) where the salt is potassium, sodium or ammonium. Also included are mixtures of the above salts.

The complexes or conjugates of the present invention contain a ligand of Formula (I). The ligands are prepared by various processes. Typical general synthetic approaches to such processes are provided by the reaction schemes given below.

In Scheme 1, the compounds of Formula (I) are prepared wherein Q, A and Z = CH, and all three R =

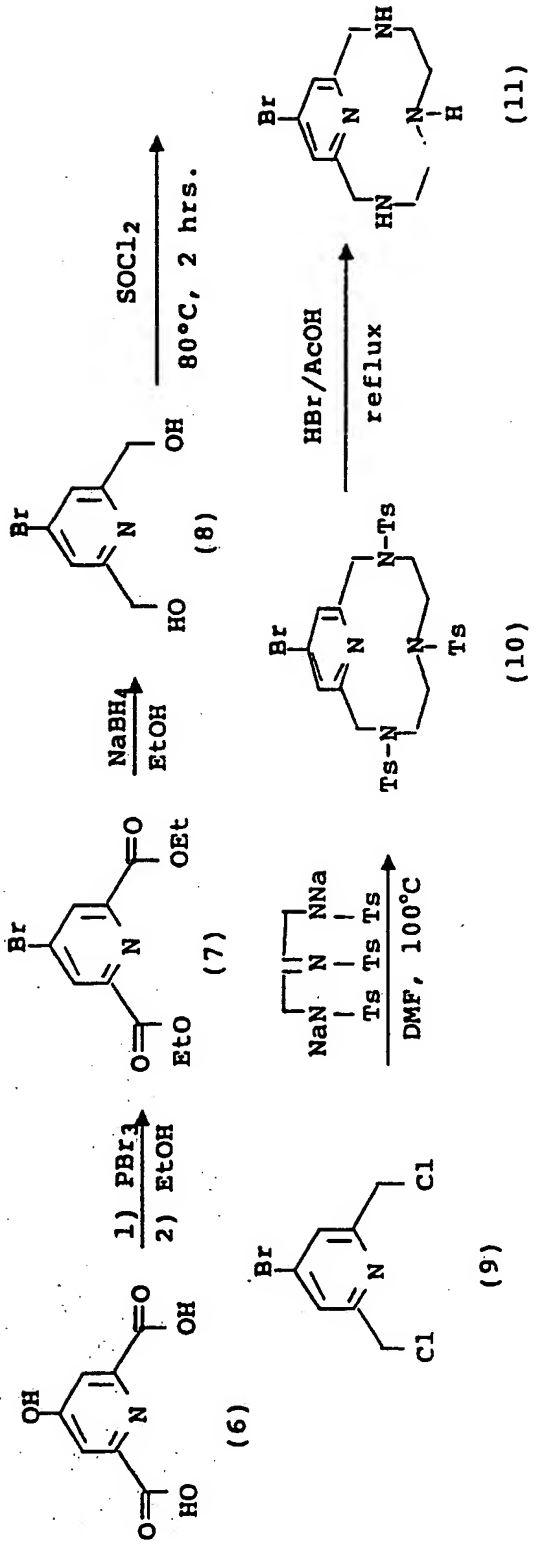


Scheme 1

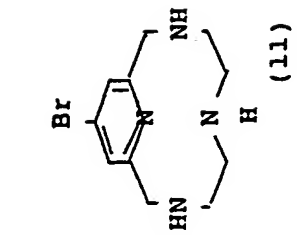

SOCl2
80°C, 2 hours
DMF, 100°C
HBr/AcOH
reflux
X-CH2-CO2H
(X=Br or Cl)
NaOH/H2O
(1)
(2)
(3)
(4)
(5)
a compound of Formula (I)

Scheme 2 prepares the compounds of Formula (I) wherein A = C-Br, and Q and Z = CH.

Scheme 2


1) PBr3
2) EtOH
NaBH4
EtOH
SOCl2
80°C, 2 hrs.
HBr/AcOH
reflux
NaN Ts Ts NNa
Ts Ts
DMF, 100°C
(6)
(7)
(8)
(9)
(10)
(11)

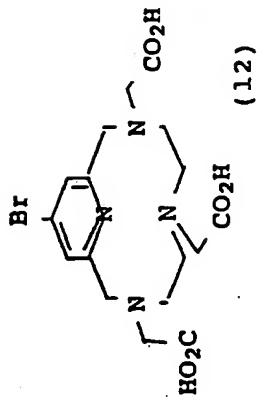
Scheme 2 Cont'd



(11)

$BrCH_2CO_2H$

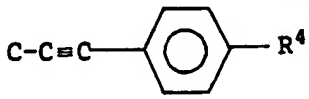
$NaOH/H_2O$



(12)

a coumpound of Formula (I)

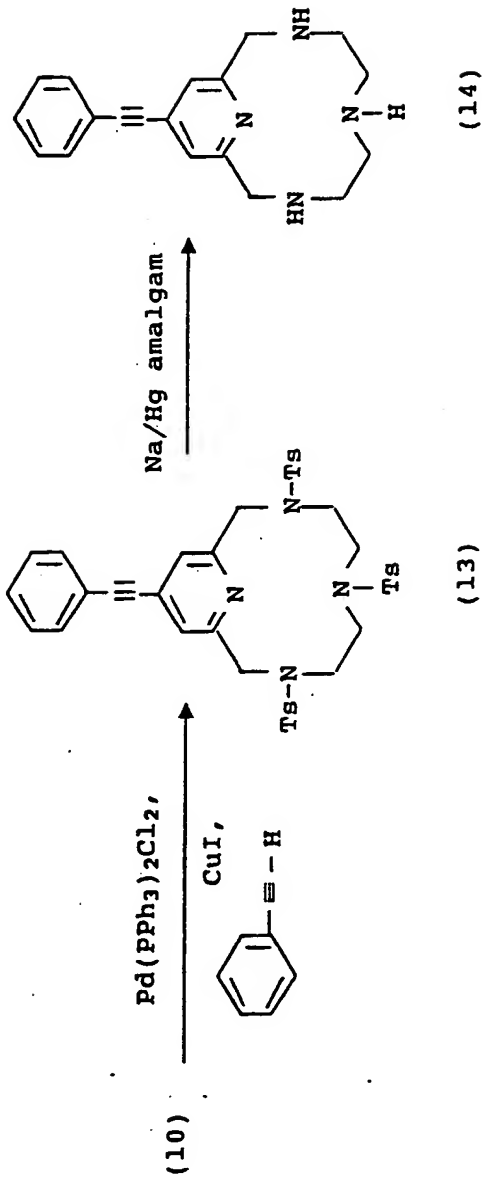
Scheme 3 prepares the compounds of Formula (I) wherein A =



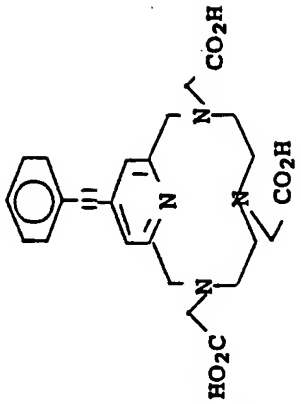
;

$R^4$ = H, $NO_2$, $NH_2$ or SCN; and Q and Z = CH.

Scheme 3



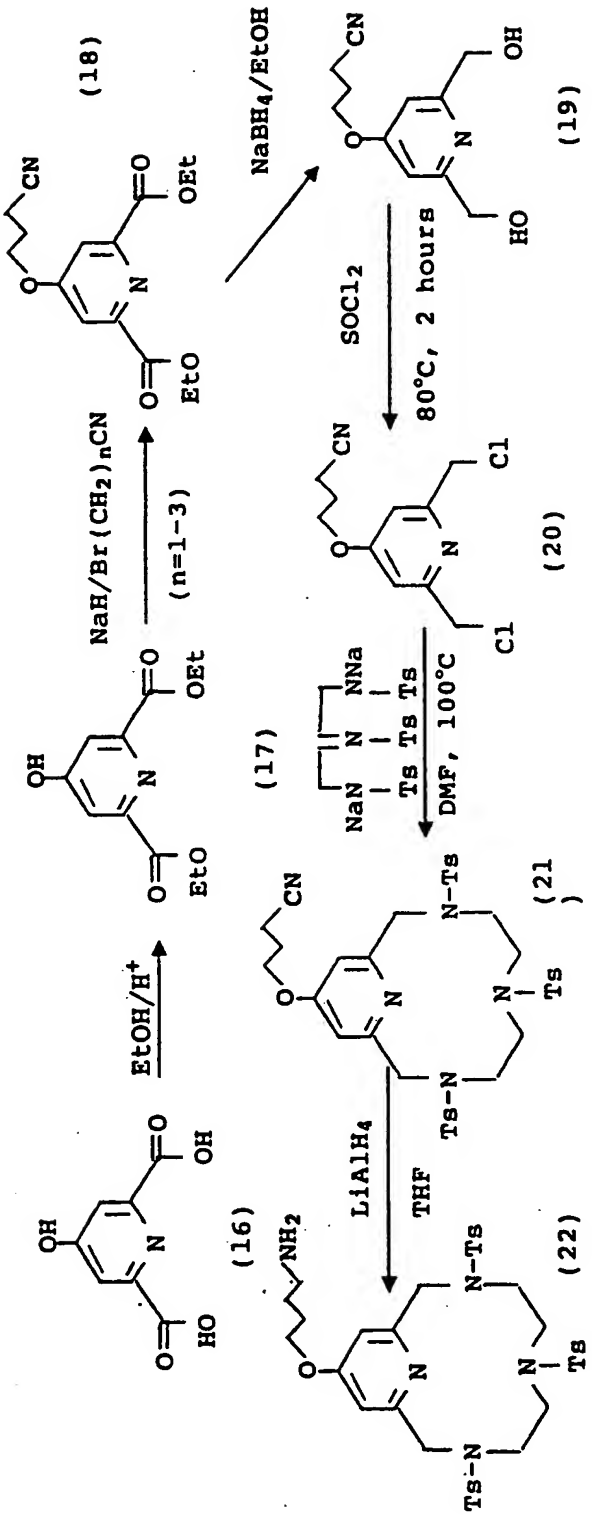
Scheme 3 Cont'd



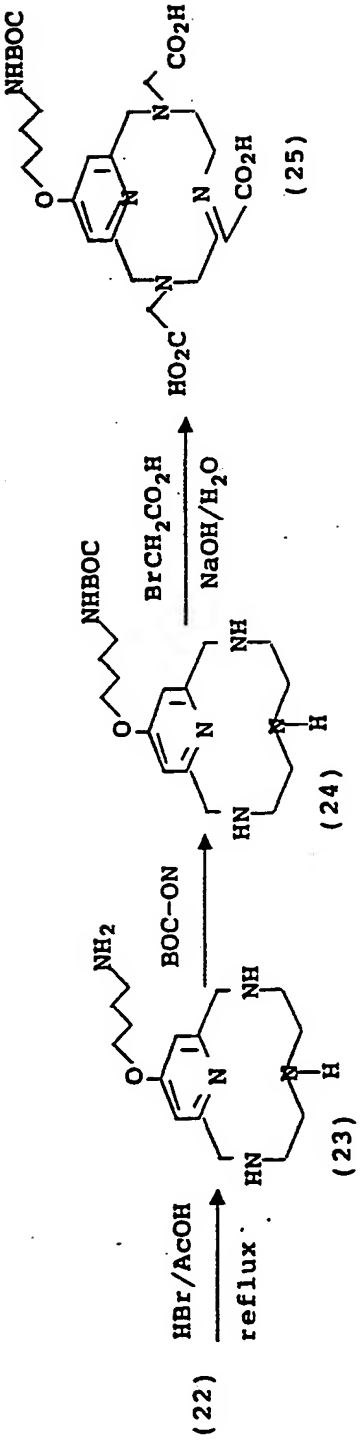

(15)
a compound of Formula (I)

Scheme 4 prepares the compounds of Formula (I) wherein A = C-OR$^2$, where R$^2$ = $C_1$-$C_5$ alkylamino; and
Q and Z = CH.

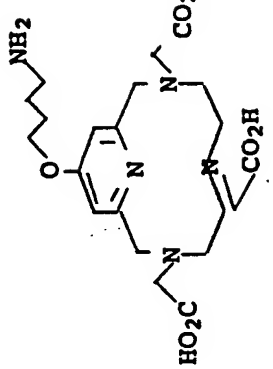
Scheme 4


(16)
(17)
(18)
(19)
(20)
(21)
(22)
EtOH/H+
NaH/Br(CH2)nCN
(n=1-3)
NaBH4/EtOH
SOCl2
80°C, 2 hours
DMF, 100°C
LiAlH4
THF

Scheme 4 Cont'd



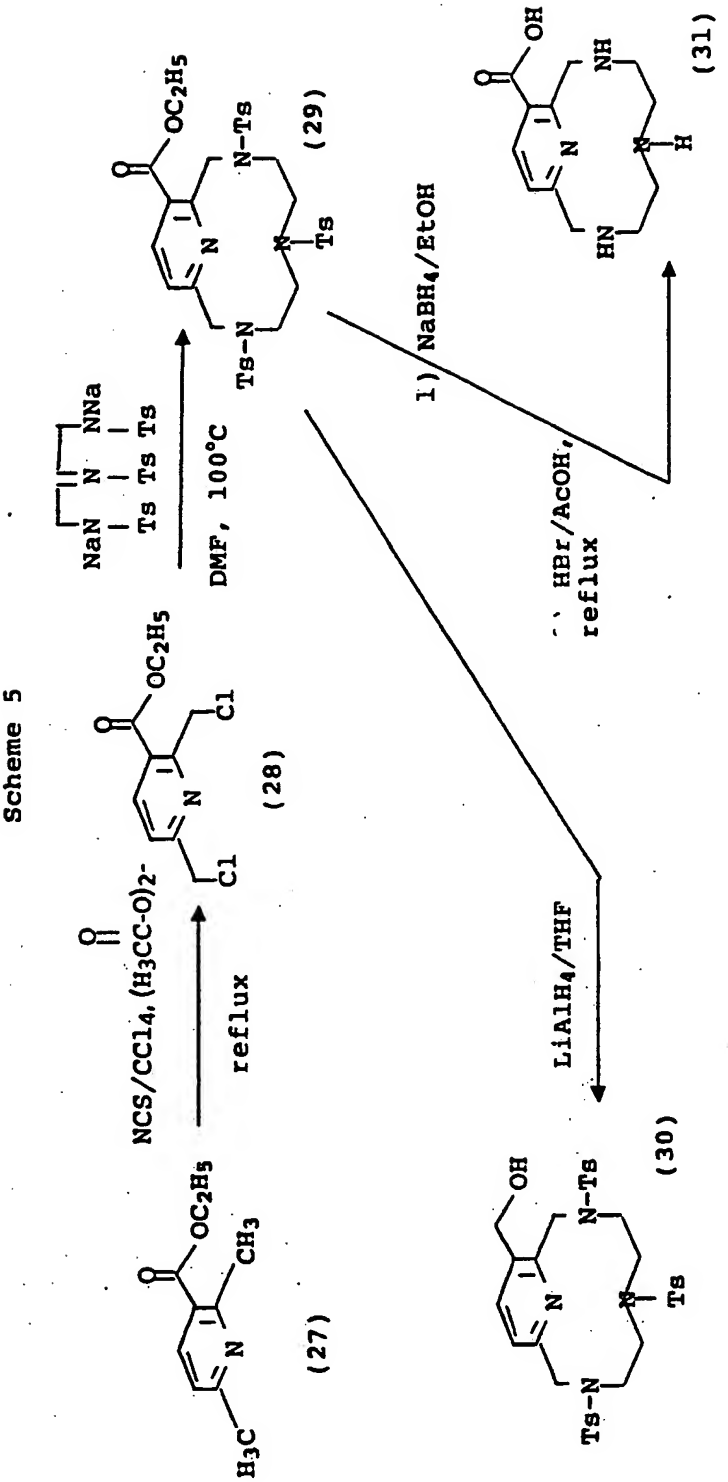
Scheme 4 Cont'd




(25) $F_3CCO_2H$ (26) a compound of Formula (I)

Scheme 5 prepares the compounds of Formula (I) wherein A = CH; and one of Q or Z = CH and the other Q or Z = C-C(O)-$R^6$ or C-$CH_2$-$R^6$, where $R^6$ is defined as before.

Scheme 5


NCS/CCl4, (H3CC-O)2-
reflux
DMF, 100°C
NaN NNa
Ts Ts Ts
1) NaBH4/EtOH
HBr/AcOH,
reflux
LiAlH4/THF
OC2H5
CH3
H3C
Cl
N-Ts
Ts
Ts-N
OH
NH
HN
H
(27)
(28)
(29)
(30)
(31)

Scheme 5 Cont'd

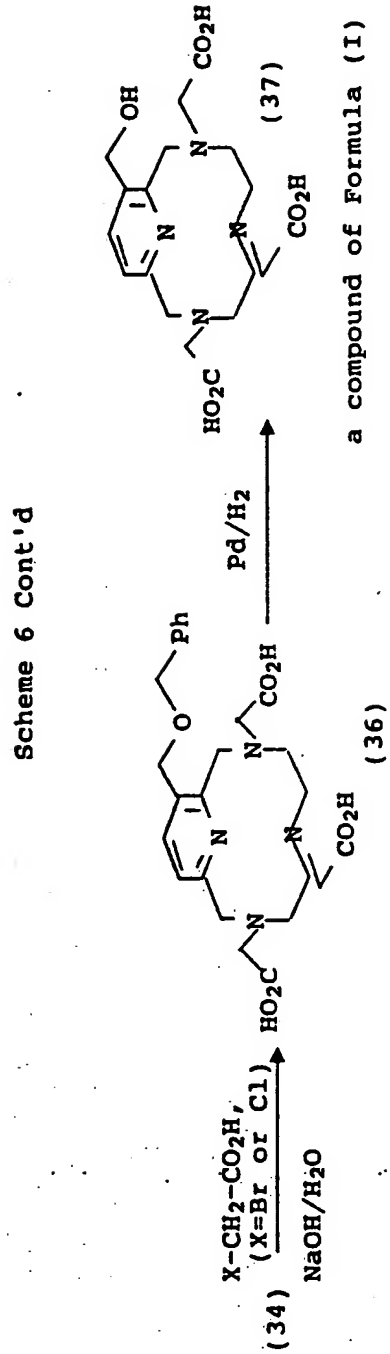



(32)

a compound of Formula (I)

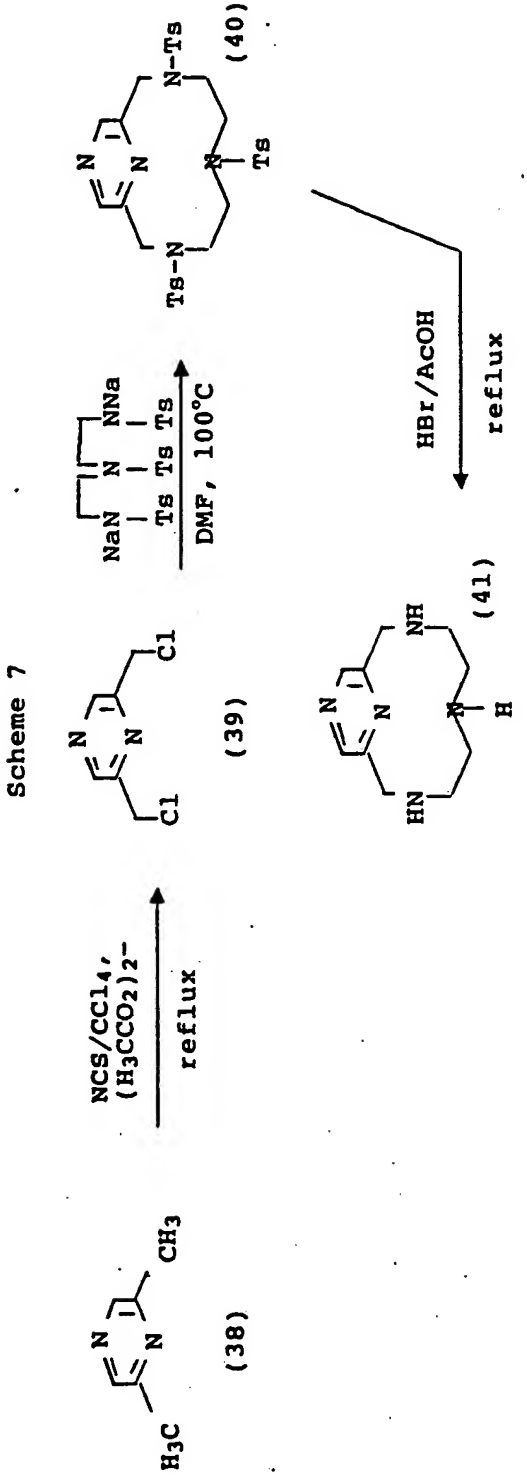
Scheme 6 prepares the compounds of Formula (I) wherein Z = C-$CH_2$-OBz or C-C(O)-$R^5$ where $R^5$ = -O-($C_1$-$C_3$ alkyl), OH or $NHR^6$, where is defined as before; and Q and A = CH.

Scheme 6 Cont'd

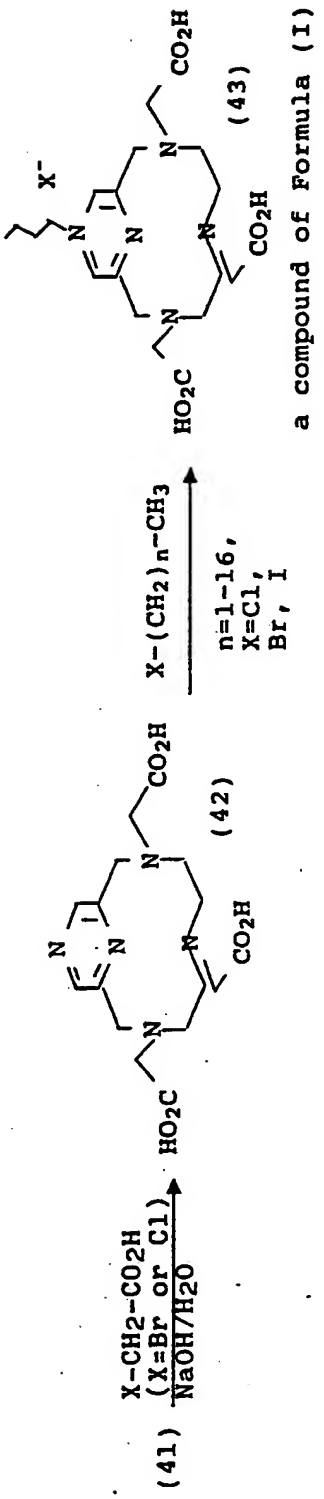


Scheme 7 prepares the compounds of Formula (I) wherein A = N or $N^+$-$R^5X^-$; $R^5$ = $C_1$-$C_{16}$ alkyl and is X halide; and
Q and Z = CH.

Scheme 7



Scheme 7 Cont'd

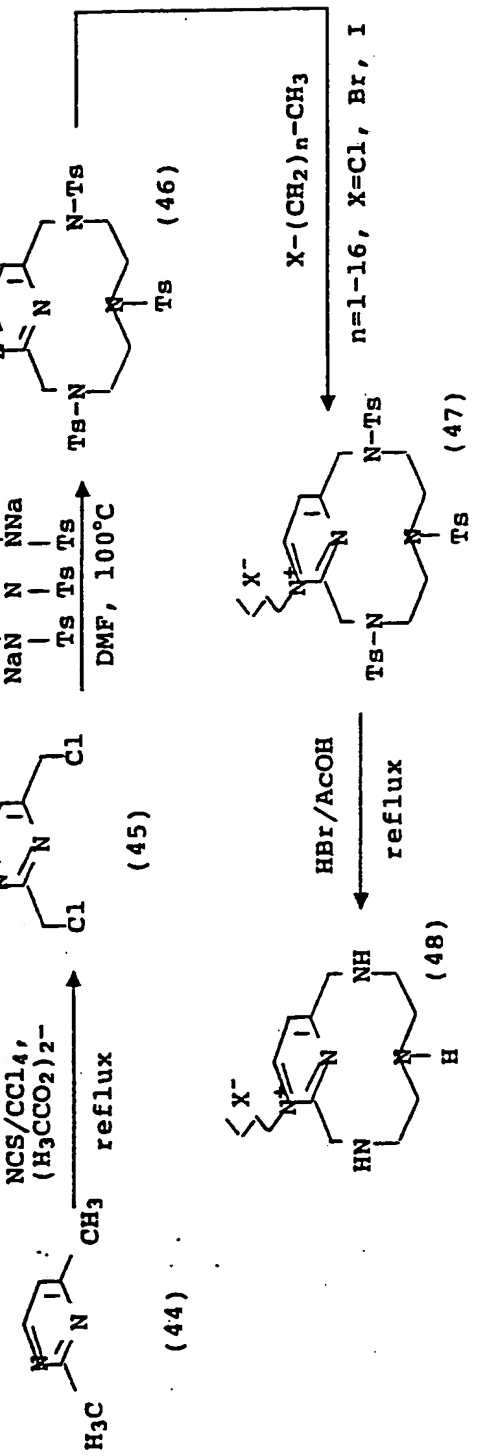


Scheme 8 prepares the compounds of Formula (I) wherein

Q = $N^+$-$R^5$ $X^-$, where $R^5$ = $C_1$-$C_{16}$ alkyl and $X^-$ = halide; and

A and Z = CH.

Scheme 8


NCS/CCl4,
(H3CCO2)2-
reflux
CH3
H3C
(44)
Cl
Cl
(45)
NaN
NNa
Ts Ts Ts
DMF, 100°C
Ts-N
N-Ts
Ts
(46)
X-(CH2)n-CH3
n=1-16, X=Cl, Br, I
X-
Ts-N
N-Ts
Ts
(47)
HBr/AcOH
reflux
X-
HN
NH
H
(48)

Scheme 8 Cont'd

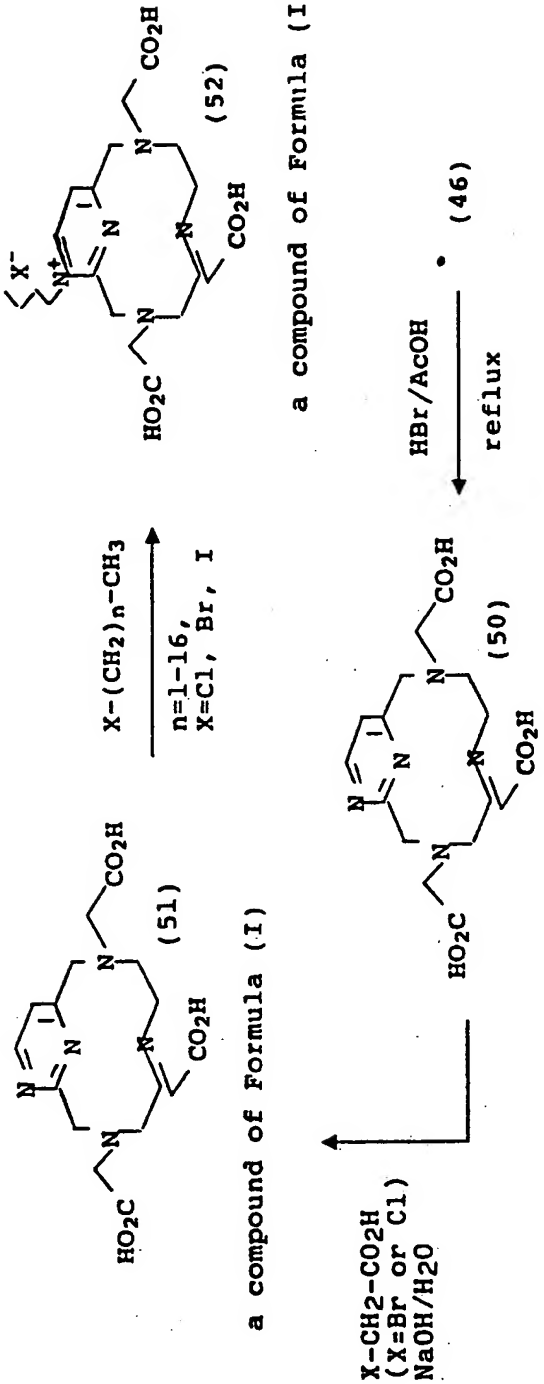
Scheme 9 prepares the compounds of Formula (I) wherein

Q = N or N+-$R^5$ $X^-$, where $R^5$ = $C_1$-$C_{16}$ alkyl and

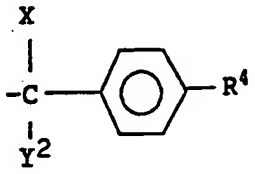
$X^-$ = halide; and

A and Z = CH.

Scheme 9


a compound of Formula (I)
HBr/AcOH
reflux
(46)
(50)
X-CH2-CO2H
(X=Br or Cl)
NaOH/H2O
a compound of Formula (I)
X-(CH2)n-CH3
n=1-16,
X=Cl, Br, I
(51)
(52)

Scheme 10 prepares the compounds of Formula (I) wherein R term at the 6 position is

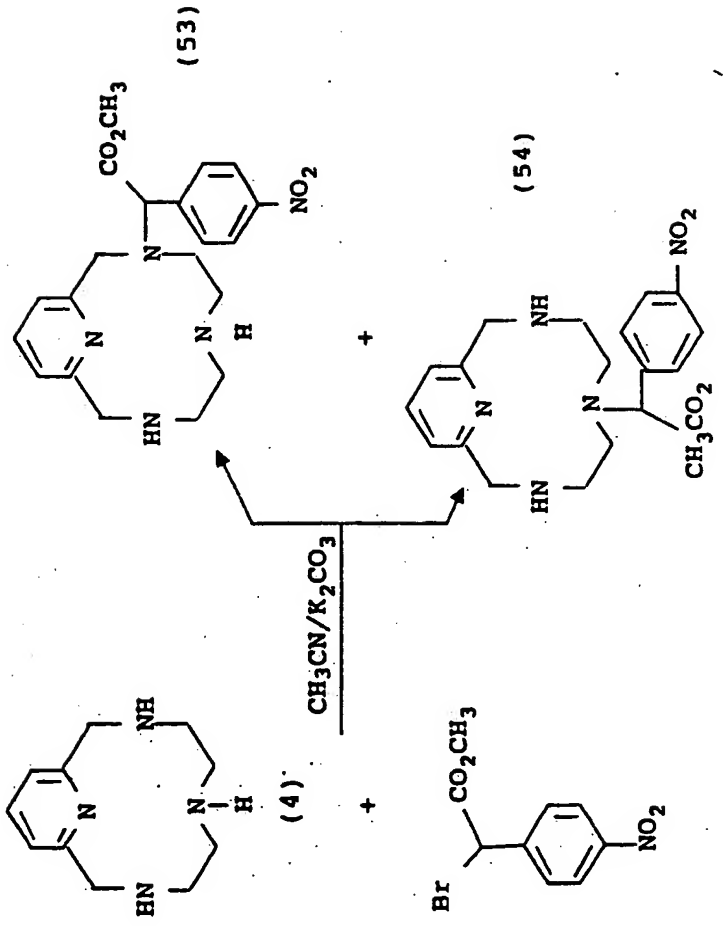


where $R^4 = NO_2$ or $NH_2$;

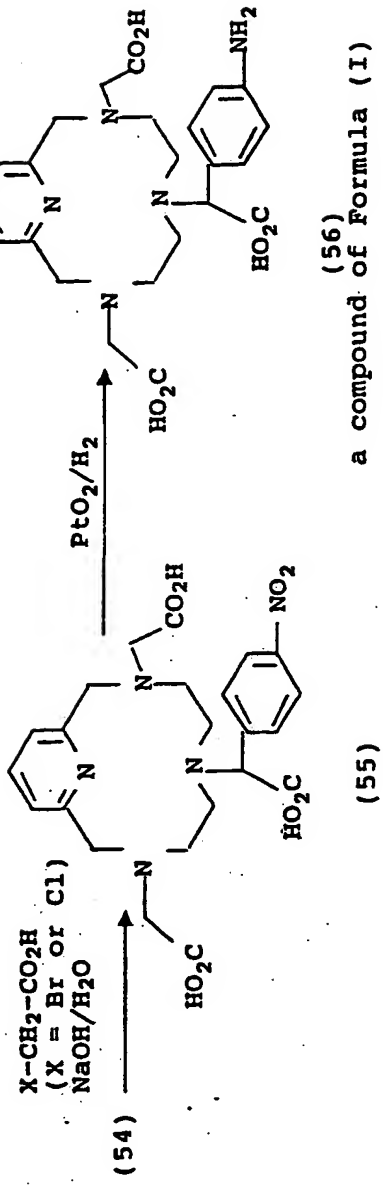
$Y^2 = CO_2H$ (or with a change of reagent $Y^2 = H$); and

A, Q and Z = CH.

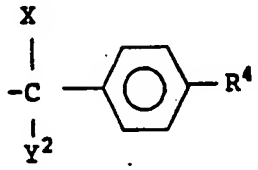
Scheme 10



Scheme 10 Cont'd

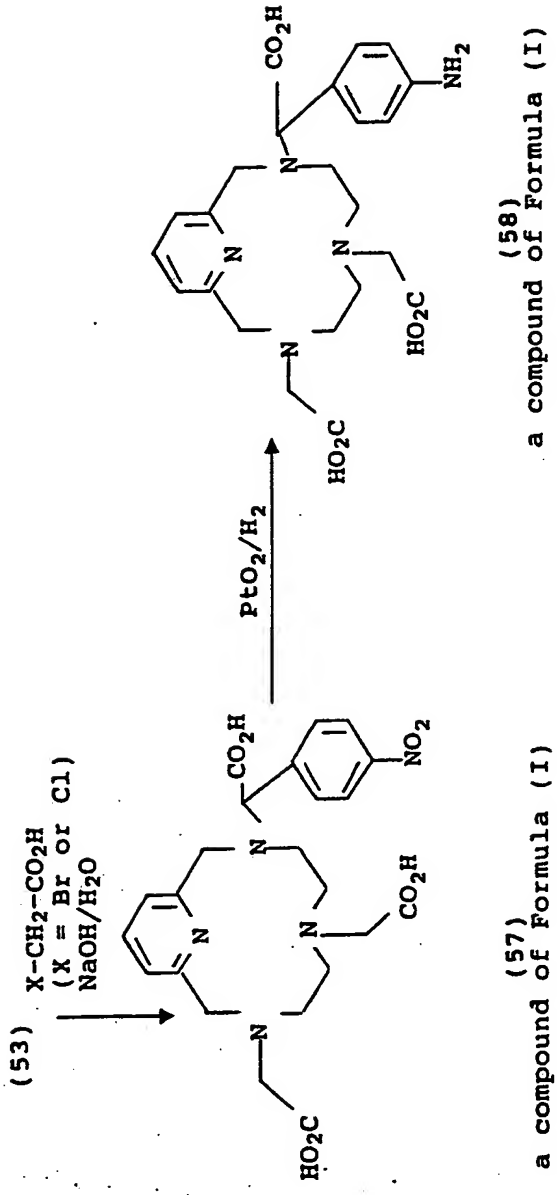


Scheme 11 prepares the compounds of Formula (I) wherein the R term at the 9 position is



where $R^4 = NO_2$ or $NH_2$;
$Y^2 = CO_2H$ (or with a change of reagent $Y^2 = H$); and
A, Q and Z = CH.

Scheme 11




(57) a compound of Formula (I)

(58) a compound of Formula (I)

Scheme 12 prepares the compounds of Formula (I) wherein n = 1 (but would also apply if n = 2 or 3 with the corresponding change in the reagent), the R term at the 6 position has T =

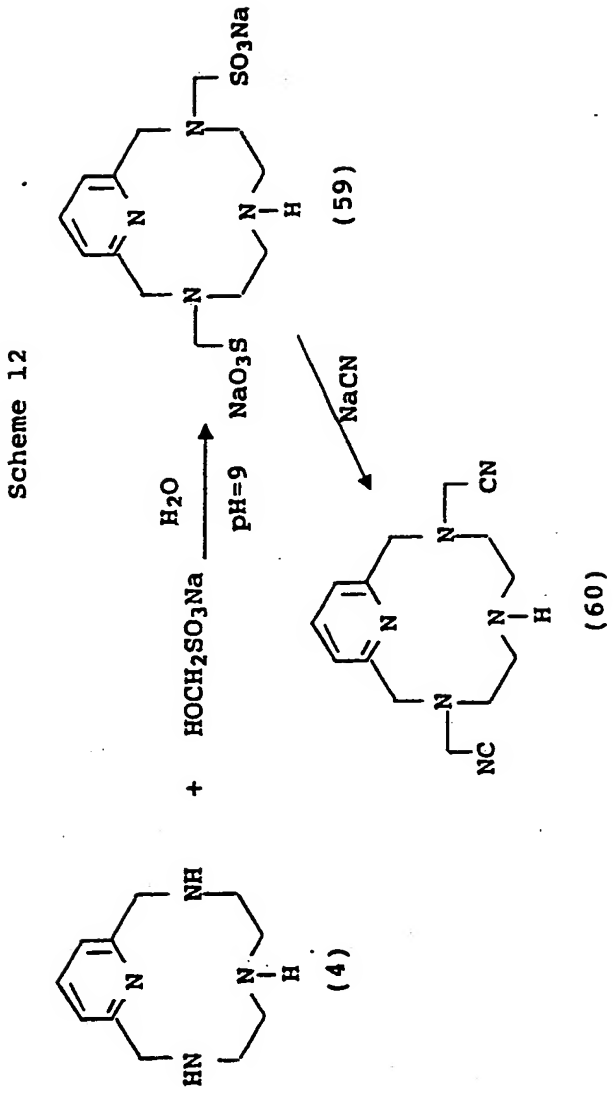
where $R^1$ = -OH; and X and Y = H;

the R term at the 3 and 9 positions have T = COOH;

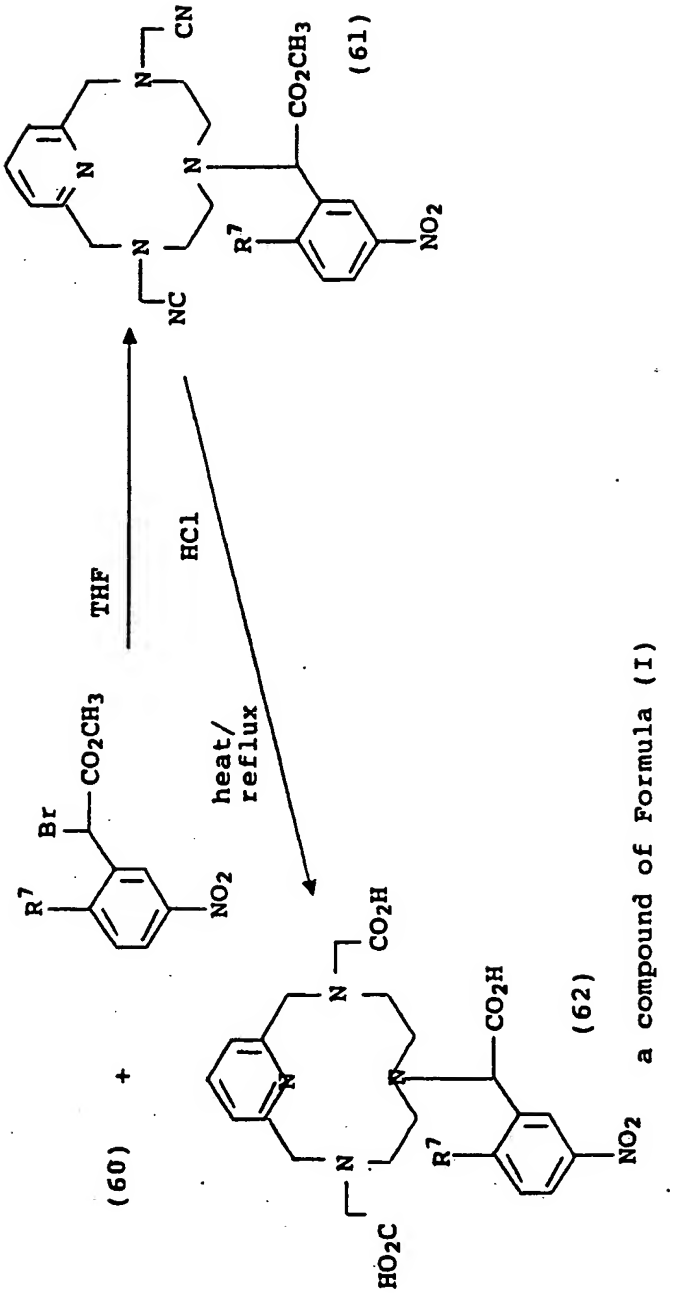
$R^7$ = OH or $OCH_3$; and

A, Q and Z = CH.

Scheme 12



Scheme 12 Cont'd




a compound of Formula (I)

In the above Schemes, the general process description illustrates specific steps that may be used to accomplish a desired reaction step. The general description of these process steps follows.

The synthetic Scheme 1 begins with a halogenation of commercially available bis-pyridyl alcohol (1) using thionyl chloride. Similar procedures for converting an alcohol to an electrophilic substrate, such as treatment with toluenesulfonyl chloride, HBr or HCl, should also result in a similarly reactive product which would work well in subsequent ring closure reactions. Macrocyclization procedures are numerous in the literature and the desired tetraazamacrocycle (3) was prepared according to the method of Stetter et al., *Tetrahedron* 37, 767-772 (1981). More general procedures have since been published which give good yields of similar macrocycles using milder conditions [A. D. Sherry et al., *J. Org. Chem.* 54, 2990-2992 (1989)]. Detosylation of the intermediate macrocycle [(3) to yield (4)] was accomplished under acidic conditions in good yield. Reductive detosylation procedures are also well known in the literature and can be adapted to the present reaction sequence.

Schemes 10, 11 and 12 delineate a synthetic approach which introduces an aromatic nitrobenzyl substituent at one of the macrocyclic nitrogen positions. Typically, the macrocyclic amine is mono-N-functionalized in an organic solvent such as acetonitrile or DMF at room temperature using a non-nucleophilic base such as potassium carbonate. Additional functionalization of the remaining nitrogen positions is then preformed by methods and conditions described in previous Schemes. After the introduction of the desired chelating moieties, the nitro group is reduced using platinum oxide and hydrogen in water. In this form, the chelating agent is compatible with conjugation techniques which will enable attachment to larger synthetic or natural molecules.

The metal ions used to form the complexes of this invention are $Gd^{+3}$, $Mn^{+2}$, $Fe^{+3}$ and available commercially, e.g. from Aldrich Chemical Company. The anion present is halide, preferably chloride, or salt free (metal oxide).

A "paramagnetic nuclide" of this invention means a metal ion which displays spin angular momentum and/or orbital angular momentum. The two types of momentum combine to give the observed paramagnetic moment in a manner that depends largely on the atoms bearing the unpaired electron and, to a lesser extent, upon the environment of such atoms. The paramagnetic nuclides found to be useful in the practice of the invention are gadolinium ($Gd^{+3}$), iron ($Fe^{+3}$) and manganese ($Mn^{+2}$), with $Gd^{+3}$ being preferred.

The complexes are prepared by methods well known in the art. Thus, for example, see Chelating Agents and Metal Chelates, Dwyer & Mellor, Academic Press (1964), Chapter 7. See also methods for making amino acids in Synthetic Production and Utilization of Amino Acids, (edited by Kameko, et al.) John Wiley & Sons (1974). An example of the preparation of a complex involves reacting a bicydopolyazamacrocyclophosphonic acid with

the metal ion under aqueous conditions at a pH from 5 to 7. The complex formed is by a chemical bond and results in a stable paramagnetic nuclide composition, e.g. stable to the disassociation of the paramagnetic nuclide from the ligand.

The complexes of the present invention are administered at a ligand to metal molar ratio of at least about 1:1, preferably from 1:1 to 3:1, more preferably from 1:1 to 1.5:1. A large excess of ligand is undesirable since uncomplexed ligand may be toxic to the animal or may result in cardiac arrest or hypocalcemic convulsions.

The antibodies or antibody fragments which may be used in the conjugates described herein can be prepared by techniques well known in the art. Highly specific monoclonal antibodies can be produced by hybridization techniques well known in the art, see for example, Kohler and Milstein [*Nature*, 256, 495-497 (1975); and *Eur. J. Immunol.*, 6, 511-519 (1976)]. Such antibodies normally have a highly specific reactivity. In the antibody targeted conjugates, antibodies directed against any desired antigen or hapten may be used. Preferably the antibodies which are used in the conjugates are monoclonal antibodies, or fragments thereof having high specificity for a desired epitope(s). Antibodies used in the present invention may be directed against, for example, tumors, bacteria, fungi, viruses, parasites, mycoplasma, differentiation and other cell membrane antigens, pathogen surface antigens, toxins, enzymes, allergens, drugs and any biologically active molecules. Some examples of antibodies or antibody fragments are 1116-NS-19-9, 1116-NS-3d, 703D4, 704A1, CC49, CC83 and B72.3. All of these antibodies have been deposited in ATCC. A more complete list of antigens can be found in U.S. Patent 4,193,983. The conjugates of the present invention are particularly preferred for the diagnosis of various cancers.

This invention is used with a physiologically acceptable carrier, excipient or vehicle therefor. The methods for preparing such formulations are well known. The formulations may be in the form of a suspension, injectable solution or other suitable formulations. Physiologically acceptable suspending media, with or without adjuvants, may be used.

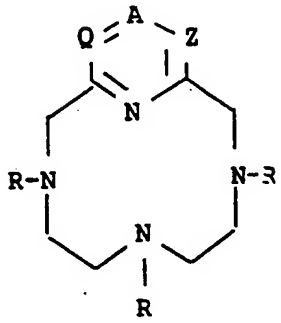
An "effective amount" of the formulation is used for diagnosis. The dose will vary depending on the disease and physical parameters of the animal, such as weight. *In vivo* diagnostics are also contemplated using formulations of this invention.

Other uses of some of the chelants of the present invention may include the removal of undesirable metals (i.e. iron) from the body, attachment to polymeric supports for various purposes, e.g. as diagnostic agents, and removal of metal ion by selective extraction. The ligands of Formula (I) having in at least two R terms T equal to $P(O)R^1OH$ may be used for metal ion control as scale inhibitors. It is likely that these ligands could be used in less than stoichiometric amounts. Similar uses are known for compounds described in U.S. Patents 2,609,390; 3,331,773; 3,336,221; and 3,434,969.

The invention will be further clarified by a consideration of the following examples, which are intended to be purely exemplary of the present invention.

WHAT IS CLAIMED IS:

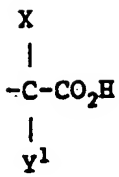
1. A complex which comprises a bicyclopolyazamacrocyclocarboxylic acid compound of the formula



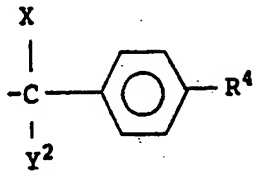
(I.

wherein:

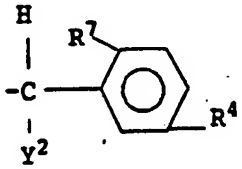
R is



,



or



;

where:

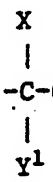
X and $Y^1$ are independently H, OH or $C_1$-$C_3$ alkyl;

$Y^2$ is H or COOH;

$R^7$ is H, OH or $OCH_3$; and

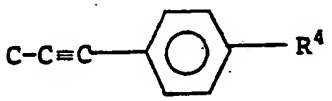
$R^4$ is H, $NO_2$, $NH_2$, isothiocyanato, semicarbazido, thiosemicarbazido, maleimido, bromoacetamido or carboxyl;

with the proviso that at least two R terms must be



;

A = CH, N, C-Br, C-Cl, C-$OR^1$, C-$OR^2$, $N^+$-$R^3$ $X^-$, or



;

where: $R^1$ = H, $C_1$-$C_5$ alkyl, benzyl, or benzyl substituted with at least one $R^4$;

$R^2$ is $C_1$-$C_{16}$ alkylamino;

$R^3$ is $C_1$-$C_{16}$ alkyl, benzyl, or benzyl substituted with at least one $R^4$;

$R^4$ is defined as before;

$X^-$ is $Cl^-$, $Br^-$, $I^-$ or $H_3CCO_2^-$;

Q and Z independently are CH, N, $N^+$-$R^3$ $X^-$, C-$CH_2$-$OR^1$ or C-C(O)-$R^5$;

$R^1$ and $R^3$ are defined as above;

$R^5$ is -O-($C_1$-$C_3$ alkyl), OH or $NHR^6$;

$R^6$ is $C_1$-$C_5$ alkyl or a biologically active material;

$X^-$ is defined as above; and

with the proviso that:

a) when Q, A or Z is N or $N^+$-$R^3$ $X^-$, then the other two groups must be CH;

b) when A is C-Br, C-Cl, C-$OR^1$ or C-$OR^2$, then both Q and Z must be CH;

c) the sum of the $R^2$, $R^4$ and $R^6$ terms, when present, may not exceed one; and

d) only one of Q or Z can be C-C(O)-$R^5$ and when one of Q or Z is C-C(O)-$R^5$, then A must be CH; and

complexed with a metal ion selected from $Gd^{+3}$, $Mn^{+2}$ or $Fe^{+3}$; or

pharmaceutically-acceptable salts thereof.

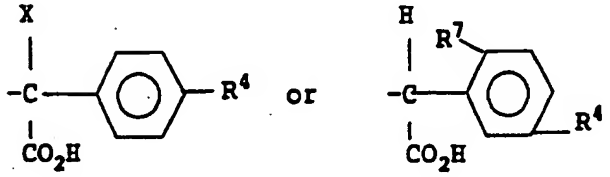
2. A complex of Claim 1 wherein the metal is $Gd^{+3}$.

3. A complex of Claim 1 wherein A, Q and Z are CH; and X and Y are H.

4. A complex of Claim 1 wherein X and Y are H.

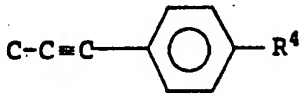
5. A complex of Claim 1 wherein A, Q and Z are CH.

6. A complex of Claim 1 wherein Q, A and Z are CH; and one R term is



where: X, Y, $R^2$ and $R^4$ are defined as in Claim 1.

7. A complex of Claim 1 wherein A is C-$OR^1$, C-$OR^2$, where $R^1$ and $R^2$ are defined as in Claim 1 or

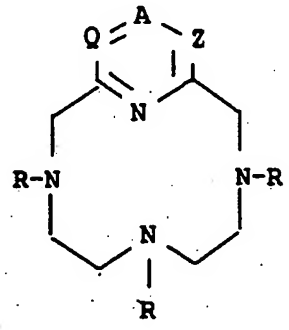


where $R^4$ is defined as in Claim 1.

8. A complex of Claim 1 wherein A is CH, and one of Q or Z is CH and the other is C-C(O)-$R^5$ or C-$CH_2$-$OR^1$, where $R^1$ and $R^5$ are defined as in Claim 1.

9. A complex of Claim 8 wherein $R^5$ is $NHR^6$, where $R^6$ is a biologically active material.

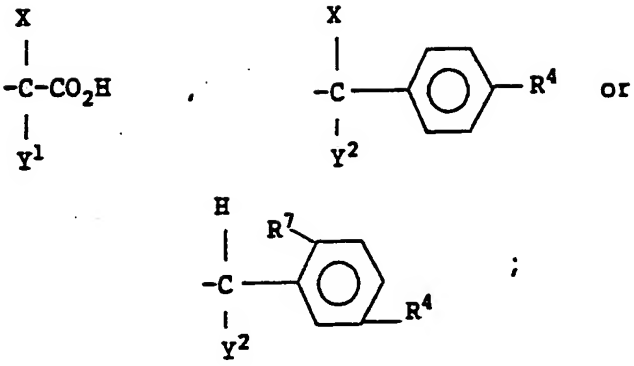
10. A conjugate comprising a bicyclopolyazamacrocyclocarboxylic acid compound of the formula



(I)

wherein:

R is



where:

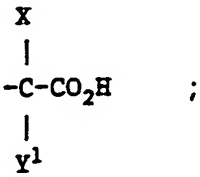
X and $Y^1$ are independently H, OH or $C_1$-$C_3$ alkyl;

$Y^2$ is H or COOH;

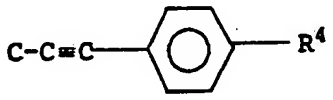
$R^7$ is H, OH or $OCH_3$; and

$R^4$ is H, $NO_2$, $NH_2$, isothiocyanato, semicarbazido, thiosemicarbazido, maleimido, bromoacetamido or carboxyl;

with the proviso that at least two R terms are



A = CH, N, C-Br, C-Cl, C-$OR^1$, C-$OR^2$, $N^+$-$R^3$ $X^-$, or



$R^1$ = H, $C_1$-$C_5$ alkyl, benzyl, or benzyl substituted with at least one $R^4$;

$R^2$ is $C_1$-$C_{16}$ alkylamino;

$R^3$ is $C_1$-$C_{16}$ alkyl, benzyl, or benzyl substituted with at least one $R^4$;

$R^4$ is defined as before;

$X^-$ is $Cl^-$, $Br^-$, $I^-$ or $H_3CCO_2^-$;

Q and Z independently are CH, N, $N^+$-$R^3$ $X^-$, C-$CH_2$-$OR^1$ or C-C(O)-$R^5$;

$R^1$ and $R^3$ are defined as above;

$R^5$ is -O-($C_1$-$C_3$ alkyl), OH or $NHR^6$;

$R^6$ is $C_1$-$C_5$ alkyl or a biologically active material;

X is defined as above; and

with the proviso that:

a) when Q, A or Z is N or $N^+$-$R^3X^-$, then the other two groups must be CH;

b) when A is C-Br, C-Cl, C-$OR^1$ or C-$OR^2$, then both Q and Z must be CH;

c) the sum of the $R^2$, $R^4$ and $R^6$ terms may not exceed one, and one of $R^2$, $R^4$ or $R^6$ must be present; and

d) only one of Q or Z can be C-C(O)-$R^5$ and when one of Q or Z is C-C(O)-$R^5$, then A must be CH;

complexed with a metal ion selected from $Gd^{+3}$, $Mn^{+2}$ or $Fe^{+3}$; and

covalently attached to a biologically active material.

11. A conjugate of Claim 10 wherein the biologically active material is a dextran, a peptide, a molecule that has specific affinity for a receptor, or an antibody or antibody fragment.

12. A conjugate of Claim 11 wherein the antibody or antibody fragment is a monoclonal antibody or fragment thereof.

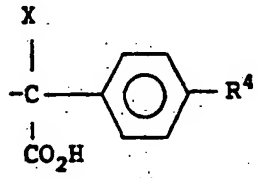
13. A conjugate of Claim 12 wherein the antibody or antibody fragment is B72.3.

14. A conjugate as claimed in any one of Claims 10-13 wherein the metal ion is $Gd^{+3}$.

15. A conjugate of Claim 10 wherein X and Y are H.

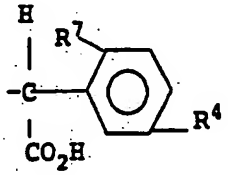
16. A conjugate of Claim 10 wherein A, Q and Z are CH.

17. A conjugate of Claim 10 wherein Q, A and Z are CH; and one R term is



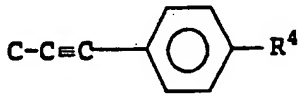
where: X and $R^4$ are defined as in Claim 10.

18. A conjugate of Claim 10 wherein Q, A and Z are CH; and one R term is



where: $R^4$ and $R^7$ are defined as in Claim 10.

19. A conjugate of Claim 10 wherein A is C-$OR^1$, C-$OR^2$, where $R^1$ and $R^2$ are defined as in Claim 10, or



where $R^4$ is defined as in Claim 10.

20. A conjugate of Claim 10 wherein A is CH, and one of Q or Z is CH and the other is C-C(O)-$R^6$, where $R^6$ is defined as in Claim 10.

21. A conjugate of Claim 20 wherein $R^6$ is $NHR^7$, where $R^7$ is a biologically active material.

22. A pharmaceutical formulation comprising a complex as claimed in any one of Claims 1-9 with a pharmaceutically-acceptable carrier.

23. A pharmaceutical formulation comprising a conjugate as claimed in any one of Claims 10-21 with a pharmaceutically-acceptable carrier.

24. A method for the diagnosis of a disease state in an animal which comprises administering to said animal an effective amount of the formulation of Claim 22.

25. A method for the diagnosis of a disease state in an animal which comprises administering to said animal an effective amount of the formulation of Claim 23.

26. The complex as claimed in any one of Claims 1-9 for use as a pharmaceutical.

27. The conjugate as claimed in any one of Claims 10-21 for use as a pharmaceutical.

28. A kit for use as a diagnostic agent having as an ingredient a ligand as claimed in any one of Claims 1-9.

29. A process for preparing a complex as claimed in Claim 1 which comprises reacting a bicyclopolyazamacrocyclocarboxylic acid compound as claimed in Claim 1 with a metal ion selected from $Gd^{+3}$, $Mn^{+2}$ or $Fe^{+3}$ under aqueous conditions at a pH from 5 to 7.

30. The process of Claim 29 wherein the bicyclopolyazamacrocyclocarboxylic acid compound is 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-tri-methylenecarboxylic acid.

# INTERNATIONAL SEARCH REPORT

PCT/US92/11003

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 49/00; C07F 11/00, 15/00, C07D 471/10; C07K 15/28

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9; 540/465,472 530/388.22, 404,405; 536/17.1, 112; 534/15,16; 536/28.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Chemical Abstracts- CAS online -structure search

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>Y | EP,A, 0,438,206 (Gries et al.) 24 July 1991 P. 4, column 1 to 40 and examples 1 to 3, and 7 to 8. | 1 to 5, 22<br>24,26 and<br>28 to 30<br>10 to 16,23<br>25,27 |
| Y | US,A, 4,963,344 (Gries et al.) 16 October 1990 column 3, lines 22 to 66 and examples 35,43,27 and 54 to 57. | 10 to 16,23<br>25,27 |
| Y | US,A, 4,933,441 (Gibby) 12 June 1990 See the entire document. | 10 to 11,23<br>25,27 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be part of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

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|---|---|
| Date of the actual completion of the international search<br>05 MARCH 1993 | Date of mailing of the international search report<br>21 APR 1993 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. NOT APPLICABLE | Authorized officer<br>EDWARD C. WARD<br>Telephone No. (703) 308-1235 |

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## INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/11003

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>Y | EP,A, 391,766 (Jackels, et al.) 10 October 1990 See example 15. | 1 to 5,22<br>24,26 and<br>28 to 30<br>10 to 15,23<br>25,27 |
| X<br>Y | EP,A, 352,218 (Platzek et al.) 24 January 1990 See examples 1,2. | 1 to 5,22,24<br>26 and<br>28 to 30<br>10 to 15,23<br>25,27 |
| X | US,A, 4,920,195 (Kankare et al.) 24 April 1990 See the entire document. | 1 to 5,7<br>10 to 18<br>and 19 to<br>30 |
| Y | EP,A, 298,939 (Kwiatkowski et al.) 11 January 1989 See page 4. | 1 to 5,22<br>24,26 and<br>28 to 30 |
| X | Stetter et al., Tetrahedron Vol. 37 pp. 767-772, 1981<br>"Darstellung And Komplexbildung Von Polyazacycloalkan-N-EssigSauren. | 1 – 5 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/11003

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/9; 540/465,472 530/388.22, 404,405; 536/17.1, 112; 534/15,16; 536/28.1